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Original article

The potential implication of eosinophil activation in the pathogenesis of childhood asthma

Background: *Asthma is now recognized as an eosinophil mediated inflammation of the airways. Pulmonary function tests are less easily performed in young children. So, measuring markers of eosinophil activation is of special importance in pediatric practice.*
Objective: *This study aimed at evaluating the role of eosinophil protein X (EPX) as a marker for assessment of asthma attack severity and control of the exacerbation.*
Methods: *Serum EPX was measured in 35 asthmatic Egyptian children aged between 7 and 10 and 35 age and sex matched healthy children using radioimmunoassay technique (RIA). Asthmatic patients were graded according to severity of the attack into mild, moderate and severe and the measurement was performed during and after the resolution of acute asthma attack. In addition, complete hemogram, total serum IgE, arterial blood gases and stool analysis were performed and peak expiratory flow rate (PEFR) was assessed in asthmatic children during and after acute exacerbation.*
Results: *Serum concentrations of EPX, total serum IgE and absolute eosinophilic count (AEC) were significantly higher in asthmatic children than healthy controls (P<0.001). Serum EPX and AEC were significantly higher in all studied groups before treatment compared to the corresponding levels of the same patients after treatment (P<0.001). Total serum IgE was significantly higher only in mild and moderate asthma before treatment (P<0.001). Serum EPX level was significantly elevated among patients with severe attacks (84.70 ± 7.18 µg/L) than those with moderate attacks (67.2 ± 5.31 µg/L) and patients with mild attacks (53.47 ± 11.47 µg/L)(P<0.001). It was negatively correlated to PEFR measurement during attacks (r=-0.75, P<0.05). A significant reduction in serum EPX and AEC was observed after the resolution and improvement of pulmonary function. Meanwhile, total serum IgE decreased after treatment with the resolution of asthma attack, yet it remained significantly higher than that of controls (P<0.001).*
Conclusion: *Our findings support the concept that EPX may be implicated in the pathogenesis of asthma and highlights its importance in monitoring the severity and control of asthma exacerbation. Hence, it might represent an objective guide of treatment efficacy.*

Key words: EPX, Childhood asthma, pathogenesis, eosinophil activation.

Nayera I. Attia, Wafaa I. Rashid*

Institute of Postgraduate Childhood Studies (Medical Department), Ain Shams University and National Research Center, Cairo-Egypt.*

Correspondence:
Dr. Nayera I. Attia
Institute of Postgraduate Childhood Studies (Medical Department), Ain Shams University
Abbassiah, Cairo, Egypt.
E-mail: nayera@hotmail.com

INTRODUCTION

Asthma is recognized as an eosinophil mediated inflammation of the airways[1]. Eosinophils are major contributors to the damage in the airways of asthmatic patients which when activated, degranulate and release granules that contain cytotoxic cationic proteins including the major basic protein, eosinophil peroxidase, eosinophil cationic protein, eosinophil protein "X" (EPX), and membrane lipid mediators. These products have deleterious effects on the airway tissues and result in destruction of mucosal and smooth muscle layers which lead to the inability to clear bronchial secretions and the production of constrictor factors that cause bronchoconstriction, increased vascular permeability and development of airway hyperresponsiveness. Also, these products influence the accumulation and maintenance of eosinophilic responses at the site of inflammation[2]. So, measuring these proteins in blood to monitor inflammation in bronchial asthma patients is of a special importance in pediatric practice because lung function tests are less easily performed in young children[3]. Several studies have been done to

measure the eosinophil granule proteins in children with bronchial asthma and the results of these studies suggest that serum markers of eosinophil activation correlate with airway function in childhood asthma and may be of value in assessing the severity of the disease[4].

The aim of this study was to evaluate the role of eosinophil protein X as a marker for assessment of asthma attack severity and control of exacerbation.

METHODS

Patients group:

This follow up case-control study was carried out on 35 Egyptian asthmatic children (20 boys and 15 girls) aged between 7 and 10 years with a mean of 8.3±1.2 years. These patients were recruited from the Outpatient Pediatric Chest Clinic, Children's Hospital, Ain Shams University during the period from January to December 2002. Patients were assessed for asthma severity according to GINA guidelines[5] and were categorized as mild (n=10), moderate (n= 15) and severe asthmatics (n= 10).

The patients were evaluated during acute asthmatic attacks and were followed up until the attack subsided clinically and then re-evaluated after 2 weeks of treatment (bronchodilators and short term inhaled steroids). Among these patients, 80% had family history of allergy and parental smoking. Exclusion criteria for the patients were: the presence of acute lower respiratory tract infection, fever, parasitic infestation (by stool analysis) and treatment with long-term corticosteroids whether inhaled or systemic.

Control group: thirty-five age and sex matched healthy children were included in this study to serve as controls. They had no personal or family history of allergic diseases.

Methods:

All the studied children were subjected to the following:

- Clinical evaluation as regards family history of atopy, the duration and severity of symptoms, records of drug therapy as well as assessment of asthma severity. PEFR was measured by a Mini-Wright peak flow meter (Clement Clarke International Ltd, Edinburgh, Essex CM202 DE, England).
- Laboratory investigations included complete hemogram including total and differential leukocytic counts (Coulter counter T660, Coultronics, France), blood gas analysis (blood gas analyzer Mod. 995, Hb-Trust Medical Company), stool analysis, total serum IgE level using ELISA technique (Medix Biotech Inc., Agenzyme Company, San Carios CA, USA).

Serum eosinophil protein X (EPX) was measured by radioimmunoassay technique (RIA)[(4)] using a commercially available kits supplied by Pharmacia Company, (100 Route 206 North Peapack, New Jersey 07977, USA).

Serum "EPX", total serum IgE, absolute eosinophilic count (AEC) and PEFR were measured twice for each asthmatic patient (during and after resolution of acute asthma attack (with a 2 weeks interval).

Sample collection:

Blood specimens were obtained under complete aseptic condition from the studied individuals by:

Arterial puncture (radial artery): 1 ml blood in heparinized syringe for pH and blood gas analysis (Kept in ice till analyzed).

Venipuncture: 1 ml was collected in a test tube containing EDTA for complete hemogram and 3ml in a plain dried tube left to clot at room temperature then centrifuged at 3000 rpm to separate serum for EPX and IgE analysis (serum was frozen at -20^{o}c till assayed).

Statistical methods:

Data processing of the results statistical analysis statistically using SSPS soft ware package (Version "8", 1997 USA). Student's t test was used to compare the mean values of parametric data and ANOVA for evaluating quality of several groups.

RESULTS

The mean serum EPX concentration, total serum IgE and absolute eosinophilic count (AEC) were significantly higher while PEFR was significantly lower in asthmatic children than in healthy controls ($P<0.001$) (table 1).

The serum EPX levels and AEC were significantly dropped whereas PEFR significantly increased with the resolution of the attacks ($P<0.001$). Total serum IgE was significantly higher only in mild and moderate asthma during the attack compared to the level in remission ($P<0.001$) (table 2).

The relationship of asthma severity to different laboratory parameters was studied by ANOVA test. This revealed a significant direct relationship with EPX, serum total IgE and AEC, whereas the relation with PEFR was a significant inverse one (fig.1).

Serum EPX correlated negatively with PEFR ($r=-0.75$, $P<0.05$) and positively correlated with AEC and the mean serum IgE level ($r=0.56$, $r=0.59$ respectively, $P<0.05$).

No significant correlation could be found between serum EPX and the duration of illness. Serum EPX did not differ significantly between patient with history of other atopic diseases (27±65µg/L) and those with no history of atopic diseases (26±27µg/L). Following treatment and resolution of acute asthma symptoms with therapy the mean serum EPX, AEC and PEFR became insignificantly differed from the control values (P>0.05). The mean total serum IgE level decreased after treatment, yet it remained significantly higher than that of the control group (P<0.001) (table 3).

Table 1: Comparison between asthmatic patients during attacks and controls as regard serum EPX, total serum IgE, AEC and PEFR.

	Control group (n=35)	**Asthmatic patients (n=35)**	**P**
	mean±SD	mean±SD	
Serum EPX (µg/L)	23.95±4.38	79.71±6.90	P<0.001
Total serum IgE (IU/ml)	34.73±7.38	270.30±160.40	P<0.001
AEC(%)	1.82±0.85	12.30±1.40	P<0.001
PEFR (%)	97.36±9.36	73.20±2.24	P<0.001

P<0.001= highly significant

Table 2: Serum EPX, total IgE, AEC and PEFR in the study groups during acute attacks and in remission.

	Mild asthmatics (n = 10)		**Moderate asthmatics (n=15)**		**Severe asthmatics (n=10)**	
	During attack	**In remission**	**During attack**	**In remission**	**During attack**	**In remission**
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Serum EPX (µg/L)	53.47 ±11.47	25.27 ±4.04	67.2 ±5.31	26.93 ±3.75	84.7 ±7.18	26.6 ±3.24
P	<0.001		<0.001		<0.001	
Total serum IgE (Iu/ml)	204.67 ±66.33	179.27 ±62.25	254.33 ±119.6	204.53 ±88.9	273.4 ±170.39	248.7 ±155.81
P	<0.05		<0.005		>0.05	
AEC (%)	10.87 ±2.29	1.73 ±0.80	11.33 ±1.68	1.91 ±0.81	12.4 ±1.58	2.0 ±0.82
P	<0.001		<0.001		<0.001	
PEFR (%)	73.20 ± 2.24	94.60 ±7.75	61.07 ±4.77	93.40 ±4.72	48.4 ±1.26	95.2 ±7.44
P	<0.001		<0.001		<0.001	

P>0.05= non significant P<0.05=significant P<0.001=highly significant

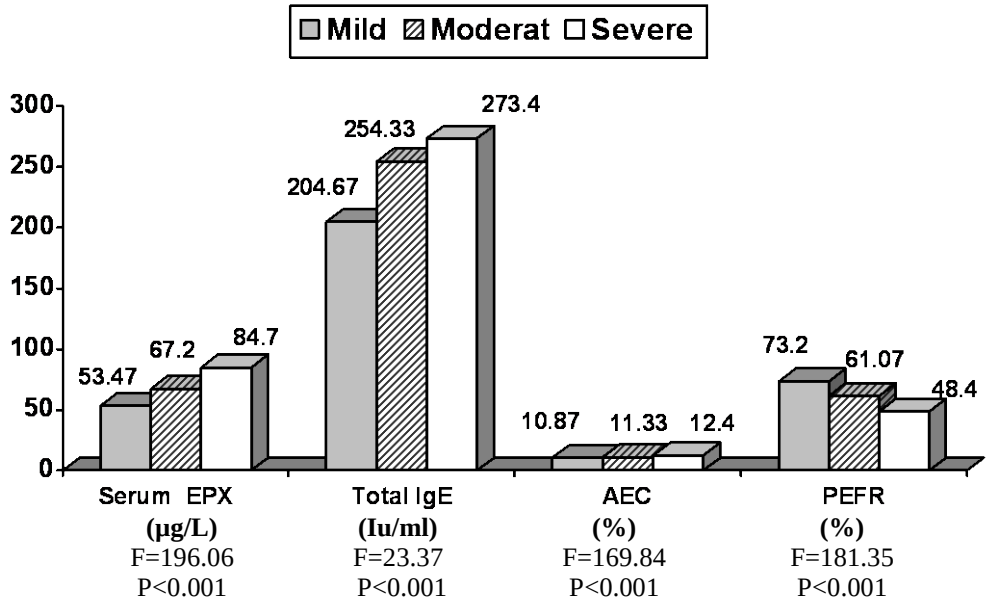

Figure 1: Relation of the laboratory findings and PEFR to the severity of asthma (P values reflect the differences among the groups by ANOVA test).

Table 3: Comparison between patients groups and controls as regards serum EPX, total serum IgE, AEC and PEFR after treatment.

Variants	Controls	Mild asthmatics (n = 10)	Moderate asthmatics (n=15)	Severe asthmatics (n=10)
	Mean ±SD	Mean ±S. D	Mean ±SD	Mean ±SD
Serum EPX (µg/L)	23.95 ±4.38	25.27 ±4.04	26.93 ±3.75	26.60 ±3.24
P		>0.05	>0.05	>0.05
Total serum IgE (Iu/ml)	34.73 ±7.38	179.27 ±62.25	204.53 ±88.9	248.70 ±155.81
P		<0.001	<0.001	<0.001
AEC (%)	1.82 ±0.85	1.80 ±0.77	1.87 ±0.74	2.0 ±0.82
P		>0.05	>0.05	>0.05
PEFR (%)	97.36 ±9.36	94.60 ±7.75	93.40 ±4.72	95.20 ±7.44
P		>0.05	>0.05	>0.05

P>0.05 = non significant , P<0.001= highly significant

DISCUSSION

Eosinophils are involved in the inflammatory response in asthma and their basic proteins play a major pathophysiological role in this process as they cause cytoxic damage to the airways epithelium in asthma. The serum levels of these basic proteins have been used to monitor the ongoing asthmatic disease. Assessment of eosinophil derived proteins in various body fluids (serum, urine and nasal lavage) could be used for monitoring disease activity of childhood asthma[7]. So, this study aimed at evaluating serum EPX as a marker for assessment of disease severity and prognosis in asthmatic children.

The results of this study revealed that 80% of patients had positive family history of allergy and parental smoking. This finding was in agreement with that of Cohen[8] who stated that 75% of asthmatic patients had a positive family of asthma or other atopic disorders. As regards parental smoking, Wright et al[9] found that parental smoking was associated with an increased risk of asthma in children.

In fact, the role of serum EPX was evident in our results since its concentrations was significantly increased in asthmatic children as compared with the control group. In this regard, Remes et al[10] and Koller et al[7] found that the level of serum EPX was significantly raised in children with bronchial asthma compared with healthy control subjects. Also, Boner et al[11] and Oymar[12] reported that levels of EPX were higher in children with asthma during acute attacks than in controls.

An important aim of this study was to evaluate serum EPX in asthmatic children during and after the resolution of an asthma attack. Serum EPX significantly increased in all asthmatic children during exacerbations compared to the values observed with the resolution of the attacks ($P<0.001$). In support of our results, Labbe et al [(1)] reported that there was significant reduction in the level of EPX in asthmatic children after treatment when compared to their corresponding value before treatment.

Another goal of this study was to evaluate whether the level of serum EPX was influenced by the severity of asthma attack. The results showed that during exacerbations of asthma the more severe the grade of asthma was, the higher was the mean serum EPX. Moreover, serum EPX correlated negatively to PEFR in asthmatic children. These findings denote that EPX production is positively correlated with the degree of bronchospasm. In agreement with our results Breuer et al[(13)] documented that the levels of EPX in serum and body fluids were correlated with disease activity and severity in atopic respiratory disease. Also, Lugosi et al[14] and Shirakawa et al[15] concluded that levels of EPX in serum and urine were negatively correlated with parameters of pulmonary function in asthmatic children. Recently, Bahceciler et al[16] stated that while EPX concentrations had increased, pulmonary function tests had decreased significantly in asthmatic children during acute attacks.

It is of value also to document that with resolution of the attacks, the levels of EPX became insignificantly different from controls. This could be a reflection of decreased recruited eosinophilis to the site of allergic inflammation. Therefore, it could be a useful marker in the follow up of disease severity and activity. In agreement with our results Vila et al[17] reported that eosinophil products including EPX have been related to bronchial inflammatory reactions and their levels have been shown to decline after treatment and the resolution of an asthma attack. Also, Storm et al[18] stated that measurement of serum EPX is helpful in monitoring therapy in asthmatic children. Moreover, Labbe et al[1] found that EPX levels were higher in children with asthma than in controls and after treatment there were significant reduction in the level of EPX in these asthmatic children, so, they concluded that EPX levels were sensitive and useful to the clinician in the evaluation of manifestations of airway inflammation in asthmatic children.

On studying serum IgE level in all asthmatics during and after resolution of an asthma attack, it was found that it increased with increased severity of the disease and in comparison to controls. Similarly, Boner et al.[11] found that there was significant increase in the level of serum IgE in asthmatic children during acute attacks. Also, Khadadah et al[19] recorded that total serum IgE was elevated above 200 IU/L in 63% of asthmatic compared to controls.

The results of this study showed that the AEC was increased with increasing asthma severity and also it was higher during the attack compared to the count after the resolution and to controls. Lastly, no significant difference between the three studied groups after treatment and controls as regards the mean value of AEC. In agreement with our results Alvarez et al[20] and Oymer et al[12] found that the AEC was higher in asthmatics than in controls. Jang et al[2] found that moderate and severe asthmatics had significantly higher eosinophilic counts compared with mild asthmatics. Finally, Metso et al[21] stated that the AEC was decreased in asthmatics after treatment compared with controls. In the current study, PEFR decreased significantly in patients as compared to healthy controls and in all studied groups during the attack compared to the corresponding values after the resolution. Also, it decreased significantly with increased asthma severity and it increased with improvement of the clinical condition after treatment Bahceciler et al[16] found that PEFR decreased in asthmatics during acute attacks and improved (increased) with improvement of their condition.

In conclusion, serum EPX increases with increased acute asthma severity and correlates negatively with PEFR. Its levels are higher during asthma exacerbations and declines towards normality with the resolution of the attacks. Therefore, EPX may be implicated in the pathogenesis of asthma and reflect the degree of severity of asthma. This highlights the importance of EPX as an inflammatory marker detecting both the evolution and the control of asthma exacerbation. Hence, it might represent an objective guide of treatment efficacy.

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